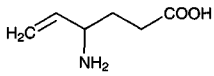
Vigabatrin



Molecular formula: $C_6H_{11}NO_2$
Molecular weight: 129.16
CAS Registry No.: 60643-86-9
Merck Index: 10114

SAMPLE

Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 100 μg/mL in MeOH:water 10:90 + 1 mL MeOH, vortex, centrifuge at 200 g. Mix 2 volumes of supernatant with 1 volume of reagent, let stand for 1 min, inject a 10 μL aliquot. (Reagent was 10 mL 30 mg/mL o-phthaldialdehyde and 200 μL 2-mercaptoethanol made up to 50 mL with 400 mM pH 9.5 borate buffer.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Microsorb C18
Mobile phase: MeCN:MeOH:10 mM orthophosphoric acid 30:10:60
Flow rate: 2
Injection volume: 10
Detector: F ex 370 em 418-700 (filter)

CHROMATOGRAM

Retention time: 5.6
Internal standard: gamma-phenyl-gamma-aminobutyric acid (13.1)
Limit of detection: 80 ng/mL
Limit of quantitation: 540 ng/mL

OTHER SUBSTANCES

Noninterfering: carbamazepine, carbamazepine epoxide, ethosuximide, phenobarbital, phenytoin, primidone, valproic acid

KEY WORDS

serum; derivatization

REFERENCE

Tsanaclis,L.M.; Wicks,J.; Williams,J.; Richens,A. Determination of vigabatrin in plasma by reversed-phase high-performance liquid chromatography, *Ther.Drug Monit.*, **1991**, *13*, 251–253.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 500 μL IS solution + 1 mL MeCN, vortex for 5 min, centrifuge for 15 min. Mix 6 μL buffer, 6 μL reagent, and 6 μL supernatant, let stand for 1 min, inject the whole amount. (Prepare IS solution by dissolving 100 mg gamma-phenyl-gamma-aminobutyric acid and 10 mg 1-(aminomethyl)cycloheptane acetic acid in 500 mL MeCN and 500 mL water. Prepare buffer by dissolving 15.5 mg boric acid in 500 mL water and adjusting to pH 9.5 with concentrated NaOH. Prepare reagent by mixing 100 mg o-phthaldialdehyde, 9 mL MeOH, 1 mL buffer, and 100 μL mercaptoethanol.)

HPLC VARIABLES

Column: 250 × 4 5 μm BANsil C18 (ASMT, Enger, Germany)
Mobile phase: Gradient. A was MeCN:MeOH:0.1% pH 2 phosphoric acid 10:10:80. B was MeCN:MeOH 50:50. A:B 90:10 for 1 min, to 30:70 over 25 min, maintain at 30:70 for 3 min, return to initial conditions over 0.1 min, re-equilibrate for 3.9 min.
Column temperature: 40
Flow rate: 1
Injection volume: 18
Detector: F ex 235 em 435

CHROMATOGRAM

Retention time: 19.9

Internal standard: gamma-phenyl-gamma-aminobutyric acid (Marion Merrel Dow) (23.4), 1-(aminomethyl)cycloheptane acetic acid (Gö-3609, Parke Davis) (28.3)
Limit of detection: 100 ng/mL
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Extracted: gabapentin

KEY WORDS
derivatization; serum; degas mobile phase continuously with helium

REFERENCE
Juergens,U.H.; May,T.W.; Rambeck,B. Simultaneous HPLC determination of vigabatrin and gabapentin in serum with automated pre-injection derivatization, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1459–1471.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 50 μL serum or urine (diluted between 1:50 and 1:200) to 1 mL MeOH containing 3.4 μg γ-phenyl-γ-amino-n-butyric acid, vortex for 15 s, centrifuge at 2000 g for 10 min, mix 6 μL of the supernatant with 3 μL reagent, inject an aliquot. (Reagent was 10 mL 30 mg/mL o-phthaldialdehyde and 200 μL 2-mercaptoethanol made up to 50 mL with 400 mM pH 9.5 borate buffer (Ther. Drug Monit. 1991, 13, 251).)

HPLC VARIABLES
Column: 125 × 3 5 μm Superspher 60 RP-Select B (Merck)
Mobile phase: Gradient. A was MeCN. B was 20 mM KH_2PO_4 buffer. A:B from 22:78 to 37:63 in 12 min, from 37:63 to 55:45 in 6 min, from 55:45 to 80:20 in 1.5 min, maintain at 80:20 for 2 min
Column temperature: 35
Flow rate: 0.7
Detector: F ex 230 em 455

CHROMATOGRAM
Retention time: 10.3
Internal standard: γ-phenyl-γ-amino-n-butyric acid (14.8)
Limit of detection: 500 nM

OTHER SUBSTANCES
Extracted: gabapentin

KEY WORDS
derivatization; serum

REFERENCE
Wad,N.; Krämer,G. Sensitive high-performance liquid chromatographic method with fluorometric detection for the simultaneous determination of gabapentin and vigabatrin in serum and urine, *J.Chromatogr.B*, **1998**, *705*, 154–158.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 20 μL 400 μg/mL IS in water + 200 μL MeCN + 100 μL 30 μM copper chloride, centrifuge at 800 g for 15 min. Remove the supernatant and add it to 200 μL buffer 1 and 200 μL 2 mg/mL dansyl chloride in MeCN, vortex for 5 s, heat at 50° for 15 min, cool to room temperature, wash with 1 mL diethyl ether. Extract the aqueous phase with ethyl acetate. Wash the ethyl acetate layer with 1 mL water. Evaporate the ethyl acetate layer to dryness under a stream of nitrogen at 35°, reconstitute the residue in 2-4 mL mobile phase, inject a 50-100 μL aliquot. Urine. 10 μL Urine + 100 μL water + 20 μL 400 μg/mL IS in water + 200 μL MeCN + 100 μL 15 μM copper chloride, vortex for 5 s, add 200 μL buffer 2, add 200 μL 2 mg/mL dansyl chloride in MeCN, vortex for 5 s, heat at 50° for 15 min, cool to room temperature, wash with 1 mL diethyl ether. Extract the aqueous phase with ethyl acetate. Wash the ethyl acetate layer with 1 mL water. Evaporate the ethyl acetate layer to

dryness under a stream of nitrogen at 35°, reconstitute the residue in 2-4 mL mobile phase, inject a 50-100 μL aliquot. (Buffer 1 was 25 mL 200 mM boric acid and 20 mL 50 mM sodium borate made up to 100 mL, pH 8.45 ± 0.05. Buffer 2 was 50 mL 400 mM boric acid and 20 mL 125 mM sodium borate made up to 100 mL, pH 8.05 ± 0.05.)

HPLC VARIABLES
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: MeCN:dioxane:500 mM orthophosphoric acid 35:15:50 (Caution! Dioxane is a carcinogen!)
Flow rate: 1
Injection volume: 50-100
Detector: F ex 345 em 418 (cut-off filter)

CHROMATOGRAM
Retention time: 8.8
Internal standard: gamma-aminobenzenebutanoic acid (13.5)
Limit of detection: 10 μg/mL (urine), 500 ng/mL (plasma)

KEY WORDS
plasma; derivatization; dog; pharmacokinetics

REFERENCE
Smithers,J.A.; Lang,J.F.; Okerholm,R.A. Quantitative analysis of vigabatrin in plasma and urine by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *341*, 232–238.

SAMPLE
Matrix: bulk
Sample preparation: 2.6 mg Vigabatrin + 250 μL 200 mM sodium bicarbonate, stir for 2 min, add 250 μL 65 mg/mL N-(tert-butoxycarbonyl)-L-leucine-N-hydroxysuccinimide ester (N-t-Boc-L-leucine N-hydroxysuccinimide ester) in THF, stir for 30 min, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL trifluoroacetic acid, let stand at room temperature for 5 min, evaporate to dryness under a stream of nitrogen, reconstitute with 10 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-8
Mobile phase: MeCN:50 mM pH 7 phosphate buffer 4:96
Flow rate: 2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 4 (R-(-)), 17.5 (S-(+))
Limit of detection: 0.1% (of major enantiomer)

KEY WORDS
chiral; derivatization

REFERENCE
Chen,T.-M.; Contario,J.J. High-performance liquid chromatographic resolution of enantiomers of γ-vinyl-γ-aminobutyric acid, *J.Chromatogr.*, **1984**, *314*, 495–498.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablet to a fine powder, add 90 mL mobile phase, stir for 10 min, make up to 100 mL with mobile phase, mix thoroughly, filter (Whatman GF/F). Remove a 10 mL aliquot of the filtrate and make up to 25 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil SCX
Mobile phase: MeCN:MeOH:25 mM pH 2.8 potassium phosphate buffer 0.4:4:100

Flow rate: 1.5
Injection volume: 20
Detector: UV 210

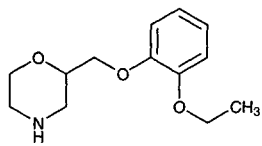
CHROMATOGRAM
Retention time: 6.4

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
tablets; stability-indicating

REFERENCE
Chen,T.-M.; Contario,J.J.; Fike,R.R. High-performance liquid chromatographic assay for vigabatrin and its primary degradation product in a pharmaceutical tablet formulation, *J.Chromatogr.*, **1987**, *398*, 351–354.

Viloxazine



Molecular formula: $C_{13}H_{19}NO_3$
Molecular weight: 237.30
CAS Registry No.: 46817-91-8, 35604-67-2 (HCl)
Merck Index: 10116
Lednicer No.: 2 306

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 222

CHROMATOGRAM
Retention time: 4.49
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam;

tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 10.993

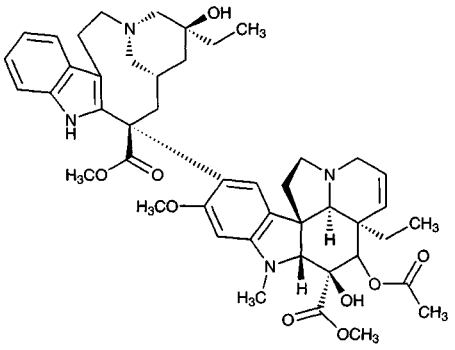
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Vinblastine



Molecular formula: $C_{46}H_{58}N_4O_9$
Molecular weight: 810.99
CAS Registry No.: 865-21-4, 143-67-9 (sulfate)
Merck Index: 10119

SAMPLE

Matrix: blood

Sample preparation: 1.2 mL Serum or plasma + 100 μL water, mix, centrifuge at 1500 g for 5 min, inject a 1 mL aliquot of the clear supernatant on to column A with mobile phase A and elute to waste, after 10 min backflush the contents of column A on to column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 3 30-38 μm pellicular ODS (Whatman) (replace daily); B 100 × 4.6 3 μm Microspher C18 (Chrompack)

Mobile phase: A MeOH:water 5:95; B MeCN:MeOH:25 mM pH 7.0 phosphate buffer 20:48:32

Flow rate: 1.25

Injection volume: 1000

Detector: UV 300 or E, ANTEC VT-03, wall-jet glassy carbon working electrode + 0.83 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 4.9

Internal standard: vinblastine

OTHER SUBSTANCES

Extracted: vincristine

KEY WORDS

serum; plasma; column-switching; human; cow; vinblastine is IS

REFERENCE

Bloemhof,H.; Van Dijk,K.N.; De Graaf,S.S.N.; Vendrig,D.E.M.M.; Uges,D.R.A. Sensitive method for the determination of vincristine in human serum by high-performance liquid chromatography after on-line column-extraction, *J.Chromatogr.*, **1991**, *572*, 171–179.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 268

CHROMATOGRAM

Retention time: 5.75
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 6 mL ether, agitate slowly for 45 min, centrifuge. Remove the organic layer and evaporate it to 1 mL, add 220 μL pH 2.7 phosphate buffer, agitate slowly for 45 min, inject a 200 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 300 × 3.9 Novapak C18
Mobile phase: MeCN:buffer 60:40 (Buffer 75 mM pH 2.70 phosphate buffer containing 100 mg/mL sodium dodecyl sulfate.)
Flow rate: 0.9
Injection volume: 200
Detector: F ex 280 em 360

CHROMATOGRAM
Internal standard: vinblastine

OTHER SUBSTANCES
Extracted: vinorelbine

KEY WORDS
plasma; vinblastine is IS

REFERENCE
Robieux,I.; Sorio,R.; Borsatti,E.; Cannizzaro,R.; Vitali,V.; Aita,P.; Freschi,A.; Galligoni,E.; Monfardini,S. Pharmacokinetics of vinorelbine in patients with liver metastases, *Clin.Pharmacol.Ther.*, **1996**, *59*, 32–40.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 8 mL diethyl ether, agitate slowly for 45 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to 1 mL, add 220 μL 75 mM pH 2.7 phosphate buffer, agitate slowly for 45 min, inject a 200 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: Novapak C18
Column: 300 × 3.9 Novapak C18
Mobile phase: MeCN:buffer 60:40 (Prepare buffer by mixing 75 mM phosphoric acid and 75 mM KH_2PO_4 in a 5:1 ratio, add sodium dodecyl sulfate to a final concentration of 100 mg/L, adjust pH to 2.80 with 2.5 M phosphoric acid.)
Flow rate: 0.9
Injection volume: 200
Detector: F ex 280 em 360

CHROMATOGRAM
Retention time: 8
Internal standard: vinblastine

OTHER SUBSTANCES
Extracted: vinorelbine

KEY WORDS
plasma; vinblastine is IS

REFERENCE
Robieux,I.; Vitali,V.; Aita,P.; Freschi,A.; Lazzarini,R.; Sorio,R. Sensitive high-performance liquid chromatographic method with fluorescence detection for measurement of vinorelbine plasma concentrations, *J.Chromatogr.B*, **1996**, *675*, 183–187.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Serum or urine + 1 mL 66 mM pH 7 phosphate buffer + 3 (serum) or 5 (urine) mL diethyl ether, rotate at 20 rpm for 30 min, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 120 μL MeOH:pH 2 HCl 20:80, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm cyano (SGE)
Mobile phase: MeCN:water 55:45 containing 40 mM ammonium acetate, pH adjusted to 3 with HCl
Flow rate: 1
Injection volume: 50
Detector: UV 268

CHROMATOGRAM
Retention time: 4.4

Internal standard: vinblastine

OTHER SUBSTANCES
Extracted: vinorelbine (navelbine)
Noninterfering: aminoglycoside antibiotics, analgesics, carbamazepine, digitoxin, furosemide, glycopeptide antibiotics, β-lactam antibiotics, phenytoin, quinidine, quinolone antibiotics, salicylic acid, theophylline

KEY WORDS
vinblastine is IS; serum

REFERENCE
Jehl,F.; Debs,J.; Herlin,C.; Quoix,E.; Gallion,C.; Monteil,H. Determination of navelbine and desacetylnavelbine in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *525*, 225–233.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL MeOH + 8 mL diethyl ether, shake for 10 min, centrifuge at 900 g for 10 min, freeze at -20° for 45 min. Remove 7 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 35-40°, reconstitute the residue in 250 μL mobile phase, wash twice with 1 mL hexane, inject a 100 μL aliquot of the aqueous phase. Urine. Centrifuge urine at 900 g. 1 mL Urine + 100 μL MeOH + 500 μL 100 mM pH 7.0 NaH_2PO_4 + 8 mL diethyl ether, shake for 10 min, centrifuge at 900 g for 10 min, freeze at -20° for 45 min. Remove 7 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 35-40°, reconstitute the residue in 250 μL mobile phase, wash twice with 1 mL hexane, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:buffer 8:50:42 (Buffer was 0.1% NaH_2PO_4 containing 400 mg/L heptanesulfonate and 300 mg/L EDTA, adjusted to pH 3.0 with 1 M phosphoric acid.)
Flow rate: 1.1
Injection volume: 100
Detector: E, Chromatofield Model Eldec 103, glassy carbon electrode 0.93 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 4.5
Internal standard: vinblastine

OTHER SUBSTANCES
Extracted: vinorelbine (navelbine)
Simultaneous: acetaminophen, aminophylline, amiodarone, carbocysteine, dextropropoxyphene, diclofenac, doxycycline, glafenine, indomethacin, morphine, noramidopyrine, propoxyphene
Noninterfering: acetylcysteine, albuterol, alizapride, amitriptyline, amoxicillin, aspirin, bromazepam, bromhexine, caffeine, clavulanic acid, clomipramine, clorazepate, diazepam, diprophylline, floctafenine, loperamide, lorazepam, methylprednisolone, metoclopramide, prednisolone, ranitidine, theophylline

KEY WORDS
vinblastine is IS; plasma

REFERENCE
Nicot,G.; Lachatre,G.; Marquet,P.; Bonnaud,F.; Vallette,J.P.; Rocca,J.-L. High-performance liquid chromatographic determination of navelbine in human plasma and urine, *J.Chromatogr.*, **1990**, *528*, 258–266.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or urine + 10 μL 10 μg/mL vintriptol in MeCN + 2.5 mL 500 mM pH 4.0 phosphate buffer + 5 mL chloroform, mix for 10 min, centrifuge at 2500 g for

10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL MeCN, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 2 5 μm Spherisorb Si
Mobile phase: MeCN:10 mM pH 3.0 citrate buffer 85:15 containing 10 mM tetrabutylammonium bromide
Flow rate: 0.2
Injection volume: 80
Detector: F ex 270 em 320 (long-pass filter)

CHROMATOGRAM
Retention time: 9
Internal standard: vintriptol (8)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
van Tellingen,O.; Beijnen,J.H.; Baurain,R.; ten Bokkel Huinink,W.W.; van der Woude,H.R.; Nooyen,W.J. High-performance liquid chromatographic determination of vinblastine, 4-O-deacetylvinblastine and the potential metabolite 4-O-deacetylvinblastine-3-oic acid in biological fluids, *J.Chromatogr.*, **1991**, *553*, 47–53.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 8.37

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cells
Sample preparation: Centrifuge cell suspensions at 200 g for 10 min, wash the pellets twice with 2 mL portions of phosphate-buffered saline, add 200 μL EtOH acidified to pH 5.5 with sulfuric acid, vortex for 2 min, centrifuge at 3000 g for 10 min, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 4 μm Novapak C18 + 150 × 3.9 4 μm Novapak C18 in series
Mobile phase: MeCN:buffer 60:40 containing 100 mg/L sodium dodecyl sulfate (Buffer was 25 mM phosphate adjusted to pH 2.7 with phosphoric acid.)
Flow rate: 0.9
Injection volume: 25
Detector: F ex 280 em 360 or UV 268

CHROMATOGRAM
Retention time: 5.6
Internal standard: vinblastine
Limit of detection: 12 pmole (UV), 2 pmole (F)

OTHER SUBSTANCES
Extracted: vinorelbine
Noninterfering: acetaminophen, aclacinomycin, amoxicillin, aspirin, daunorubicin, doxorubicin, doxycycline, heparin, insulin, methotrexate, noramidopyrine, rifamycin

KEY WORDS
vinblastine is IS

REFERENCE
Debal,V.; Morjani,H.; Millot,J.-M.; Angiboust,J.-F.; Gourdier,B.; Manfait,M. Determination of vinorelbine (Navelbine) in tumour cells by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *581*, 93–99.

SAMPLE
Matrix: plants
Sample preparation: Freeze leaves with liquid nitrogen, air dry, grind to a fine powder. Mix 0.5 g powder and 2 mL MeOH, sonicate for 30 min, allow to settle, decant the liquid, repeat extraction. Combine the extracts and filter (0.45 μm) them, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Microsorb C18
Mobile phase: Gradient. MeCN:buffer 15:85 for 2 min, to 40:60 over 58 min, maintain at 40:60 for 5 min, to 95:5 over 5 min, maintain at 95:5 over 5 min. (Prepare buffer by mixing 2 mL trifluoroacetic acid and 1 mL triethylamine in water, make up to 1 L with water, adjust pH to 2.4 with ammonium hydroxide.)
Injection volume: 10
Detector: UV 274

CHROMATOGRAM
Retention time: 45.8

OTHER SUBSTANCES
Extracted: ajmalacine, tetrahydroalstonine, tryptamine, vincamine, vincristine, yohimbine

KEY WORDS
leaves

REFERENCE
Bowman,R.N.; Gerber,R.E.; Terry,M.E. Analysis of anti-cancer alkaloids vincristine & vinblastine, *Rainin Chromatography Update (TB-13)*, **1996**, 1–2.

SAMPLE
Matrix: tissue

Sample preparation: Lyophilize tissue for 24 h, pulverize in a mortar, mix. Weigh out 25-50 mg tissue, add 5 mL 100 mM HCl, sonicate for 30 min, add 5 mL MeCN dropwise with continual vortexing, centrifuge for 30 min. Remove the supernatant and evaporate it under a stream of nitrogen at 60° to remove MeCN, add 10 mL buffer, ad 5 mL chloroform, shake for 30 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 500 μL dichloromethane, inject a 100 μL aliquot. (Buffer was 400 mM pH 3 phosphate buffer containing 50 mM octylsulfate. Silanize all glassware with Surfasil (Pierce).)

HPLC VARIABLES

Guard column: 30 × 4 5 μm LiChrosorb CN
Column: 250 × 4 5 μm LiChrosorb CN
Mobile phase: MeCN:40 mM pH 3 phosphate buffer 60:40
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM

Retention time: 19.14
Limit of detection: 10 ng/g

OTHER SUBSTANCES

Extracted: vincristine

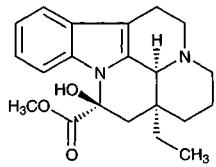
KEY WORDS

heart; kidney; lung; liver; muscle; tumor; mouse; pharmacokinetics

REFERENCE

Van Belle,S.J.-P.; de Smet,M.; De Neve,W.; Monsaert,C.; Storme,G.A.; Massart,D.L. Determination of vinca alkaloids in mouse tissues by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *578*, 223–229.

Vincamine



Molecular formula: $C_{21}H_{26}N_2O_3$
Molecular weight: 354.45
CAS Registry No.: 1617-90-9, 10592-03-7 (HCl)
Merck Index: 10120

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 221.6

CHROMATOGRAM
Retention time: 12.08

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, sal-

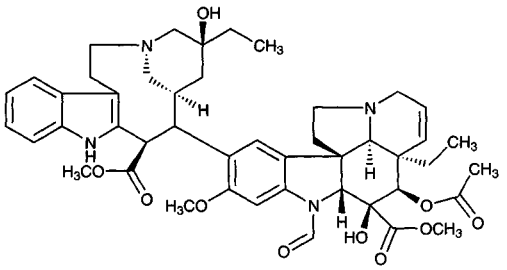
icylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Vincristine

Molecular formula: $C_{46}H_{56}N_4O_{10}$
Molecular weight: 824.97
CAS Registry No.: 57-22-7, 2068-78-2 (sulfate)
Merck Index: 10124



SAMPLE

Matrix: blood
Sample preparation: 1.2 mL Serum or plasma + 100 µL 2 µg/mL vinblastine in water, mix, centrifuge at 1500 g for 5 min, inject a 1 mL aliquot of the clear supernatant on to column A with mobile phase A and elute to waste, after 10 min backflush the contents of column A on to column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 3 30-38 µm pellicular ODS (Whatman) (replace daily); B 100 × 4.6 3 µm Microspher C18 (Chrompack)
Mobile phase: A MeOH:water 5:95; B MeCN:MeOH:25 mM pH 7.0 phosphate buffer 20:48:32
Flow rate: 1.25
Injection volume: 1000
Detector: UV 300 or E, ANTEC VT-03, wall-jet glassy carbon working electrode + 0.83 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 3.7
Internal standard: vinblastine (4.9)
Limit of detection: 1 ng/mL (UV), 0.3 ng/mL (E)

KEY WORDS

serum; plasma; column-switching; human; cow; pharmacokinetics

REFERENCE

Bloemhof,H.; Van Dijk,K.N.; De Graaf,S.S.N.; Vendrig,D.E.M.M.; Uges,D.R.A. Sensitive method for the determination of vincristine in human serum by high-performance liquid chromatography after on-line column-extraction, *J.Chromatogr.*, **1991**, *572*, 171–179.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute

the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 221

CHROMATOGRAM

Retention time: 5.06
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, cells
Sample preparation: Plasma. Add 8 mL MeCN dropwise with continuous vortexing to 4 mL plasma, centrifuge for 30 min. Remove the supernatant and evaporate it to dryness under a

stream of nitrogen at 60°, reconstitute the residue in 10 mL buffer and 5 mL chloroform, shake for 30 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 200 μL dichloromethane, inject a 100 μL aliquot. Cells. Centrifuge cell suspension, discard supernatant, add 4 mL 75 mM KCl to pellet, heat at 37° for 30 min, sonicate for 1 min, add 8 mL MeCN dropwise with continuous vortexing, centrifuge for 30 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 10 mL buffer and 5 mL chloroform, shake for 30 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 200 μL dichloromethane, inject a 100 μL aliquot. (Buffer was 400 mM pH 3 phosphate buffer containing 50 mM octylsulfate. Silanize all glassware with Surfasil (Pierce).)

HPLC VARIABLES
Guard column: 30 × 4 5 μm LiChrosorb CN
Column: 250 × 4 5 μm LiChrosorb CN
Mobile phase: MeCN:120 mM pH 3 phosphate buffer 60:40
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: 15
Internal standard: vincristine

OTHER SUBSTANCES
Extracted: vinorelbine (navelbine)
Simultaneous: vinblastine, vindesine

KEY WORDS
plasma; vincristine is IS

REFERENCE
Van Belle,S.J.-P.; de Smet,M.; Monsaert,C.; Geerts,F.; Storme,G.A.; Massart,D.L. High-performance liquid chromatographic determination of navelbine in MO_4 mouse fibrosarcoma cells and biological fluids, *J.Chromatogr.*, **1992**, *576*, 351–357.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

CHROMATOGRAM
Retention time: 13.765

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cells
Sample preparation: Add 20 μL 33% silver nitrate solution to a suspension of 2×10^6 cells, agitate for 10 s, sonicate for 20 min (Bransonic 52, Vel, Belgium), add 140 μL MeCN, vortex for 5 min, cool at 4° for 30 min, centrifuge at 10000 g for 30 s, add 200 μL 200 mM pH 3 phosphate buffer, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Hibar LiChrocart RP 18 (Merck)
Mobile phase: MeCN:buffer 35:65 (Buffer was 200 mM KH_2PO_4 containing 0.2% triethylamine, adjusted to pH 3.0 with 200 mM orthophosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 237

CHROMATOGRAM
Retention time: 4.9
Internal standard: daunorubicin (4.0)
Limit of detection: 4 pmol
Limit of quantitation: 13 pmol

OTHER SUBSTANCES
Extracted: altretamine, doxorubicin, verapamil, S 9788

KEY WORDS
human; cells; epidermoid carcinoma

REFERENCE
Tassin,J.P.; Dubois,J.; Atassi,G.; Hanocq,M. Simultaneous determination of cytotoxic (adriamycin, vincristine) and modulator of resistance (verapamil, S 9788) drugs in human cells by high-performance liquid chromatography and ultraviolet detection, *J.Chromatogr.B*, **1997**, *691*, 449–456.

SAMPLE
Matrix: formulations
Sample preparation: Inject an aliquot directly.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil 100-5CN
Mobile phase: MeCN:MeOH:20 mM pH 4.5 ammonium dihydrogen phosphate in water 20:20:60, containing 10 mM sodium heptanesulfonate
Flow rate: 1.0
Injection volume: 25
Detector: UV 297

CHROMATOGRAM
Retention time: 11.8

OTHER SUBSTANCES
Simultaneous: degradation products, doxorubicin, methylparaben, propylparaben

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Nyhammar,E.K.; Johansson,S.G.; Seiving,B.E. Stability of doxorubicin hydrochloride and vincristine sulfate in two portable infusion-pump reservoirs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1171–1173.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:buffer 50:50 (Buffer was 20 mM KH_2PO_4 adjusted to pH 5.4 with 1 M NaOH
Flow rate: 1
Injection volume: 20
Detector: UV 233

CHROMATOGRAM
Retention time: 14.7
Limit of detection: 90 ng/mL

OTHER SUBSTANCES
Simultaneous: methyl paraben, ondansetron, doxorubicin, degradation products

KEY WORDS
injections; saline

REFERENCE
King,D.T.; Venkateshwaran,T.G.; Stewart,J.T. HPLC determination of a vincristine, doxorubicin, and ondansetron mixture in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1994**, *17*, 1399–1411.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosorb RP8
Mobile phase: MeOH:buffer 70:30 (Prepare by adding 5 mL diethylamine to 295 mL water and making up to 1 L with MeOH.)
Flow rate: 1.75
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 4.01

OTHER SUBSTANCES
Simultaneous: granisetron

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: plants
Sample preparation: Freeze leaves with liquid nitrogen, air dry, grind to a fine powder. Mix 0.5 g powder and 2 mL MeOH, sonicate for 30 min, allow to settle, decant the liquid, repeat extraction. Combine the extracts and filter (0.45 μm) them, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Microsorb C18
Mobile phase: Gradient. MeCN:buffer 15:85 for 2 min, to 40:60 over 58 min, maintain at 40:60 for 5 min, to 95:5 over 5 min, maintain at 95:5 over 5 min. (Prepare buffer by mixing 2 mL trifluoroacetic acid and 1 mL triethylamine in water, make up to 1 L with water, adjust pH to 2.4 with ammonium hydroxide.)
Injection volume: 10
Detector: UV 274

CHROMATOGRAM
Retention time: 40.7

OTHER SUBSTANCES
Extracted: ajmalacine, tetrahydroalstonine, tryptamine, vinblastine, vincamine, yohimbine

KEY WORDS
leaves

REFERENCE
Bowman,R.N.; Gerber,R.E.; Terry,M.E. Analysis of anti-cancer alkaloids vincristine & vinblastine, *Rainin Chromatography Update (TB-13)*, **1996**, 1–2.

SAMPLE
Matrix: tissue
Sample preparation: Lyophilize tissue for 24 h, pulverize in a mortar, mix. Weigh out 25-50 mg tissue, add 5 mL 100 mM HCl, sonicate for 30 min, add 5 mL MeCN dropwise with continual vortexing, centrifuge for 30 min. Remove the supernatant and evaporate it under a stream of nitrogen at 60° to remove MeCN, add 10 mL buffer, ad 5 mL chloroform, shake for 30 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 500 μL dichloromethane, inject a 100 μL aliquot. (Buffer was 400 mM pH 3 phosphate buffer containing 50 mM octylsulfate. Silanize all glassware with Surfasil (Pierce).)

HPLC VARIABLES
Guard column: 30 × 4 5 μm LiChrosorb CN
Column: 250 × 4 5 μm LiChrosorb CN
Mobile phase: MeCN:40 mM pH 3 phosphate buffer 60:40
Flow rate: 1
Injection volume: 100
Detector: UV 220

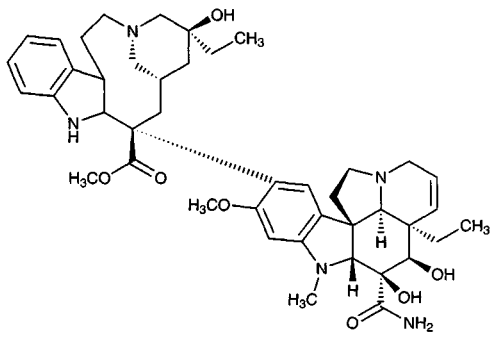
CHROMATOGRAM
Retention time: 15.60
Limit of detection: 10 ng/g

OTHER SUBSTANCES
Extracted: vinblastine

KEY WORDS
heart; kidney; lung; liver; muscle; tumor; mouse; pharmacokinetics

REFERENCE
Van Belle,S.J.-P.; de Smet,M.; De Neve,W.; Monsaert,C.; Storme,G.A.; Massart,D.L. Determination of vinca alkaloids in mouse tissues by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *578*, 223–229.

Vindesine



Molecular formula: $C_{43}H_{55}N_5O_7$
Molecular weight: 753.94
CAS Registry No.: 53643-48-4, 59917-39-4 (sulfate salt)
Merck Index: 10125

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 270

CHROMATOGRAM

Retention time: 4.92

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine;

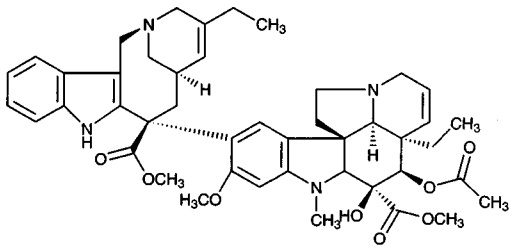
phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Vinorelbine

Molecular formula: $C_{45}H_{54}N_4O_8$
Molecular weight: 778.95
CAS Registry No.: 71486-22-1
Merck Index: 10127



SAMPLE

Matrix: blood
Sample preparation: 400 µL Plasma + 20 µL 10 µg/mL teniposide + 1.6 mL diethyl ether, vortex, centrifuge at 1000 g for 2 min. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute the residue in 200 µL mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 µm LiChrospher 100 RP-18
Mobile phase: MeCN:MeOH:buffer 30:20:50 (Buffer was 20 g/L NaH_2PO_4 containing 0.8 g/L heptanesulfonic acid, pH adjusted to 3.0 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 100
Detector: E, Environmental Sciences Coulochem 5100 A, guard cell +0.90 V (before injector), clean-up cell +0.40 V, detection cell +0.90 V

CHROMATOGRAM

Retention time: 15.5
Internal standard: teniposide (10.6)
Limit of detection: 1 ng/mL

KEY WORDS

plasma; rabbit; pharmacokinetics

REFERENCE

Mouchard-Delmas,C.; Gourdier,B.; Vistelle,R. Determination of vinorelbine in rabbit plasma by high-performance liquid chromatography with coulometric detection, *J.Chromatogr.B*, **1995**, *663*, 390–394.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 10 ng vinblastine + 6 mL ether, agitate slowly for 45 min, centrifuge. Remove the organic layer and evaporate it to 1 mL, add 220 µL pH 2.7 phosphate buffer, agitate slowly for 45 min, inject a 200 µL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 Novapak C18
Mobile phase: MeCN:buffer 60:40 (Buffer 75 mM pH 2.70 phosphate buffer containing 100 mg/mL sodium dodecyl sulfate.)
Flow rate: 0.9
Injection volume: 200
Detector: F ex 280 em 360

CHROMATOGRAM
Internal standard: vinblastine
Limit of quantitation: 2 ng/mL

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Robieux,I.; Sorio,R.; Borsatti,E.; Cannizzaro,R.; Vitali,V.; Aita,P.; Freschi,A.; Galligoni,E.; Monfardini,S. Pharmacokinetics of vinorelbine in patients with liver metastases, *Clin.Pharmacol.Ther.*, **1996**, *59*, 32–40.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 20 μL 500 ng/mL vinblastine + 8 mL diethyl ether, agitate slowly for 45 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to 1 mL, add 220 μL 75 mM pH 2.7 phosphate buffer, agitate slowly for 45 min, inject a 200 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: Novapak C18
Column: 300 × 3.9 Novapak C18
Mobile phase: MeCN:buffer 60:40 (Prepare buffer by mixing 75 mM phosphoric acid and 75 mM KH_2PO_4 in a 5:1 ratio, add sodium dodecyl sulfate to a final concentration of 100 mg/L, adjust pH to 2.80 with 2.5 M phosphoric acid.)
Flow rate: 0.9
Injection volume: 200
Detector: F ex 280 em 360

CHROMATOGRAM
Retention time: 13
Internal standard: vinblastine (8)
Limit of detection: 1 ng/mL
Limit of quantitation: 2 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Robieux,I.; Vitali,V.; Aita,P.; Freschi,A.; Lazzarini,R.; Sorio,R. Sensitive high-performance liquid chromatographic method with fluorescence detection for measurement of vinorelbine plasma concentrations, *J.Chromatogr.B*, **1996**, *675*, 183–187.

SAMPLE
Matrix: blood, cells
Sample preparation: Plasma. Add 8 mL MeCN dropwise with continuous vortexing to 4 mL plasma, centrifuge for 30 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 10 mL buffer and 5 mL chloroform, shake for 30 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 200 μL dichloromethane, inject a 100 μL aliquot. Cells. Centrifuge cell suspension, discard supernatant, add 4 mL 75 mM KCl to pellet, heat at 37° for 30 min, sonicate for 1 min, add 100 μg vincristine, add 8 mL MeCN dropwise with continuous vortexing, centrifuge for 30 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 10 mL buffer and 5 mL

chloroform, shake for 30 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 200 μL dichloromethane, inject a 100 μL aliquot. (Buffer was 400 mM pH 3 phosphate buffer containing 50 mM octylsulfate. Silanize all glassware with Surfasil (Pierce).)

HPLC VARIABLES
Guard column: 30 × 4 5 μm LiChrosorb CN
Column: 250 × 4 5 μm LiChrosorb CN
Mobile phase: MeCN:120 mM pH 3 phosphate buffer 60:40
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: 29
Internal standard: vincristine (15)
Limit of detection: 1.25 ng/mL

OTHER SUBSTANCES
Simultaneous: vinblastine, vindesine

KEY WORDS
plasma

REFERENCE
Van Belle,S.J.-P.; de Smet,M.; Monsaert,C.; Geerts,F.; Storme,G.A.; Massart,D.L. High-performance liquid chromatographic determination of navelbine in MO_4 mouse fibrosarcoma cells and biological fluids, *J.Chromatogr.*, **1992**, *576*, 351–357.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Serum or urine + 100 μL 1 μg/mL vinblastine in water, vortex, add 1 mL 66 mM pH 7 phosphate buffer, add 3 (serum) or 5 (urine) mL diethyl ether, rotate at 20 rpm for 30 min, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 120 μL MeOH:pH 2 HCl 20:80, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm cyano (SGE)
Mobile phase: MeCN:water 55:45 containing 40 mM ammonium acetate, pH adjusted to 3 with HCl
Flow rate: 1
Injection volume: 50
Detector: UV 268

CHROMATOGRAM
Retention time: 5.6
Internal standard: vinblastine (4.4)
Limit of detection: 5 ng/mL (urine), 2.5 ng/mL (serum)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: aminoglycoside antibiotics, analgesics, carbamazepine, digitoxin, furosemide, glycopeptide antibiotics, β-lactam antibiotics, phenytoin, quinidine, quinolone antibiotics, salicylic acid, theophylline

KEY WORDS
serum; pharmacokinetics

REFERENCE
Jehl,F.; Debs,J.; Herlin,C.; Quoix,E.; Gallion,C.; Monteil,H. Determination of navelbine and desacetylnavelbine in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *525*, 225–233.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 100 μL 1 μg/mL vinblastine in MeOH + 8 mL diethyl ether, shake for 10 min, centrifuge at 900 g for 10 min, freeze at -20° for 45 min. Remove 7 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 35-40°, reconstitute the residue in 250 μL mobile phase, wash twice with 1 mL hexane, inject a 100 μL aliquot of the aqueous phase. Urine. Centrifuge urine at 900 g. 1 mL Urine + 100 μL 1 μg/mL vinblastine in MeOH + 500 μL 100 mM pH 7.0 NaH_2PO_4 + 8 mL diethyl ether, shake for 10 min, centrifuge at 900 g for 10 min, freeze at -20° for 45 min. Remove 7 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 35-40°, reconstitute the residue in 250 μL mobile phase, wash twice with 1 mL hexane, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Nucleosil C18

Mobile phase: MeCN:MeOH:buffer 8:50:42 (Buffer was 0.1% NaH_2PO_4 containing 400 mg/L heptanesulfonate and 300 mg/L EDTA, adjusted to pH 3.0 with 1 M phosphoric acid.)

Flow rate: 1.1

Injection volume: 100

Detector: E, Chromatofield Model Eldec 103, glassy carbon electrode 0.93 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 6.8

Internal standard: vinblastine (4.5)

Limit of detection: 20 ng/mL (urine), 1 ng/mL (plasma)

OTHER SUBSTANCES

Simultaneous: acetaminophen, aminophylline, amiodarone, carbocysteine, dextropropoxyphene, diclofenac, doxycycline, glafenine, indomethacin, morphine, noramidopyrine, propoxyphene

Noninterfering: acetylcysteine, albuterol, alizapride, amitriptyline, amoxicillin, aspirin, bromazepam, bromhexine, caffeine, clavulanic acid, clomipramine, clorazepate, diazepam, diprophylline, floctafenine, loperamide, lorazepam, methylprednisolone, metoclopramide, prednisolone, ranitidine, theophylline

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Nicot,G.; Lachatre,G.; Marquet,P.; Bonnaud,F.; Vallette,J.P.; Rocca,J.-L. High-performance liquid chromatographic determination of navelbine in human plasma and urine, *J.Chromatogr.*, **1990**, *528*, 258–266.

SAMPLE

Matrix: blood, urine

Sample preparation: Dilute 10-20 μL urine with 500 μL blank plasma. 500 μL Plasma or diluted urine + 50 μL 1 μg/mL desacetylvinblastine in MeCN + 4 mL diethyl ether, shake vigorously for 10 min, centrifuge at 4° at 1000 g for 10 min, freeze at -20° for 1 h. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL MeCN, sonicate for 5 min, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 250 × 2 5 μm Spherisorb Si

Mobile phase: MeCN:buffer 85:15 containing 10 mM tetrabutylammonium bromide (Buffer was 10 mM trisodium citrate adjusted to pH 3.0 with HCl.)

Flow rate: 0.2

Injection volume: 80

Detector: F ex 270 em 320 (filter)

CHROMATOGRAM

Retention time: 11

Internal standard: desacetylvinblastine (17)

Limit of detection: 25 ng/mL (urine), 1.5 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
van Tellingen,O.; Kuijpers,A.; Beijnen,J.H.; Baselier,M.R.P.; Burghouts,J.T.M.; Nooyen,W.J. Bio-analysis of vinorelbine by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1992**, *573*, 328–332.

SAMPLE
Matrix: cells
Sample preparation: Centrifuge cell suspensions at 200 g for 10 min, wash the pellets twice with 2 mL portions of phosphate-buffered saline, add 20 μL 10 μM vinblastine in water, add 200 μL EtOH acidified to pH 5.5 with sulfuric acid, vortex for 2 min, centrifuge at 3000 g for 10 min, inject a 25 μL aliquot.

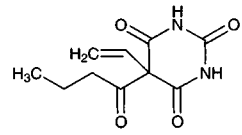
HPLC VARIABLES
Column: 300 × 3.9 4 μm Novapak C18 + 150 × 3.9 4 μm Novapak C18 in series
Mobile phase: MeCN:buffer 60:40 containing 100 mg/L sodium dodecyl sulfate (Buffer was 25 mM phosphate adjusted to pH 2.7 with phosphoric acid.)
Flow rate: 0.9
Injection volume: 25
Detector: F ex 280 em 360 or UV 268

CHROMATOGRAM
Retention time: 9.2
Internal standard: vinblastine (5.6)
Limit of detection: 13 pmole (UV), 8 pmole (F)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, aclacinomycin, amoxicillin, aspirin, daunorubicin, doxorubicin, doxycycline, heparin, insulin, methotrexate, noramidopyrine, rifamycin

REFERENCE
Debal,V.; Morjani,H.; Millot,J.-M.; Angiboust,J.-F.; Gourdier,B.; Manfait,M. Determination of vinorelbine (Navelbine) in tumour cells by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *581*, 93–99.

Vinylbital



Molecular formula: $C_{11}H_{16}N_2O_3$
Molecular weight: 224.26
CAS Registry No.: 2430-49-1
Merck Index: 10131

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

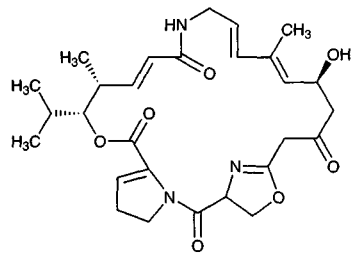
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 16.583

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Virginiamycin



Molecular formula: $C_{43}H_{49}N_7O_{10}$ (S_1), $C_{28}H_{35}N_3O_7$ (M_1)
Molecular weight: 823.91 (S_1), 525.61 (M_1)
CAS Registry No.: 21411-53-0 (M_1), 23152-29-6 (S_1)
Merck Index: 10142

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

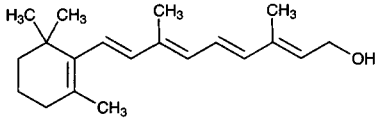
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 227.5

CHROMATOGRAM
Retention time: 17.21

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Vitamin A



Molecular formula: $C_{20}H_{30}O$
Molecular weight: 286.46
CAS Registry No.: 68-26-8
Merck Index: 10150

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 2 mL EtOH + 10 mL hexane, mix for 30 s, centrifuge at 3000 rpm for 5 min, store the hexane layer at 15°, repeat the extraction with 10 mL hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute with 200 µL isopropanol, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm TSKgel ODS-80Ts
Mobile phase: Gradient. EtOH:water 80:20 for 11.5 min then 87:13 (step gradient)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 460

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Extracted: vitamin E (F ex 298 em 325)

KEY WORDS
serum

REFERENCE
Moriyama,H.; Yamasaki,H.; Masumoto,S.; Adachi,K.; Katsura,N.; Onimaru,T. Rapid determination of vitamins A and E in serum with surfactant as a diluent by column-switching high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *798*, 125–130.

SAMPLE
Matrix: blood
Sample preparation: Dilute 100 µL serum with 900 µL 12.62 mg/mL pyrogallol in EtOH, filter (450 µm cellulose disk), cool at 15° in the autosampler, inject a 300 µL aliquot onto column A and elute to waste with mobile phase A. After 3 min backflush the contents of column A onto column B with mobile phase B, after another 1 min remove column A from the circuit. Elute column B with mobile phase B for another 7.5 min then elute with mobile phase C. Monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 4.6 13 µm TSK BSA-80Ts; B 15 × 3.2 5 µm TSK ODS-80Ts + 150 × 4.6 5 µm TSKgel ODS-80Ts
Mobile phase: A 200 mM sodium dodecyl sulfate solution:EtOH 70:30 containing 200 mM ethylenediaminetetraacetic acid 4 sodium salt and 0.3% phosphoric acid; B EtOH:water 80:20; C EtOH:water 87:13
Column temperature: 40